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PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
BRADFORD G. ADDISON
BARNES & THORNBURG LLP
11 SOUTH MERIDIAN STREET
INDIANAPOLIS, IN 46204

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*) 11 JAN 2005	
Applicant's or agent's file reference 32993-74744	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US04/08477	19 March 2004 (19.03.2004)	20 March 2003 (20.03.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): C12Q 1/54 and US Cl.: 435/14

Applicant

ADVANCED RESEARCH AND TECHNOLOGY INSTITUTE, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☐ Box No. VII — Certain defects in the international application
- ☐ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Jennifer Ione Harle Telephone No. (571) 272-1600

Form PCT/ISA/237 (cover sheet) (January 2004)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ in written format

☐ in computer readable form

c. time of filing/furnishing

☐ contained in international application as filed.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has:
   - ☐ paid additional fees
   - ☐ paid additional fees under protest
   - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
   - ☐ complied with
   - ☒ not complied with for the following reasons:

   See the lack of unity section of the International Search Report(Form PCT/ISA/210)

4. Consequently, this opinion has been established in respect of the following parts of the international application:
   - ☐ all parts.
   - ☒ the parts relating to claims Nos. 1-8

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Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	5, 7-8	YES
	Claims	1-4, 6	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-8	NO
Industrial applicability (IA)	Claims	1-8	YES
	Claims	NONE	NO

2. Citations and explanations:

Please See Continuation Sheet

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WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:
Claims 1-4 and 6 lack novelty under PCT Article 33(2) as being anticipated by Charkoudian, et al. (US 5,543,054).

Charkoudian discloses a composition for use in analyzing oligosaccarides, i.e. N-acetylglucosamine oligomers prepared as an aqueous solution (comprising a buffer), to which the oligo labeling dye ANTS (a derivatizing agent capable of forming one or more fluorescing carbohydrate derivatives from the one or mor ecarbohydres was added) and the reducing agent of soium cyanoborohydrde in dimethylsulfoxide (DMSO - a solvent) were added and incubated resulting in fluorescent band conjugateted tera-, penta-, hexa- and heptamers (carbohydrate derivatives), e and each sample was dissolved in Milli-Q water and 2X loading buffer. See col. 19, lines 1-20.

Claims 3-8 lack an inventive step under PCT Article 33(3) as being obvious over Charkoudian, et al. (5,543,054 A) in view of Wang, et al. Analysis of Chitin Oligosaccarides by Capillary Electrophoresis with Laser-Induced Fluorescence, Journal of Chromatography A, 2002, Vol. 979, pp. 431-438 and further in view of DMSO, Registry Information, November 15, 1984.

Charkoudian discloses as set forth above. However, Charkoudian does not disclose that the derivatizing agent can be 9-aminopyrene-1,4,6-trisulfonic acid, which is capable of forming one or more fluorescing carbohydrate derivates that are detectable by laser-induced fluorescence or that the buffer comprises a buffering agent selected from the group consisting of citric acid and salts there of. Wang discloses a method and the compounds utilized in the method using capillary electrophoresis (CE) with laser-induced fluorescence (LIF) detection for analyzing chitin oligosaccharides which were derivatized with 9-aminopyrene-1,4,6,trisulfonate, i.e. forms the acid in water. Abstract. Wang additionally discloses that chitin-oligosaccharides and related derivatives, which are amino polysaccharides have distinctive properties including a variety of biological activities and the fact that they are biodegradable into monomers, dimmers, trimers, tetramers, pentamers, and hexamers through ATPS derivatization. Pp. 431 and 436. Wang further discloses that carbohydrates generally do not contain chromophoric or fluorphoric groups and, as a result the determination of these compounds can be challenging, however, CE has attracted considerable amount of attention because of its high sensitivity, rapid analysis time, and high resolution and when used in conjunction with indirect detection, i.e. LIF due to its inherently high sensitivity/good specificity/and a large linear dynamic range , is a universal method and can be used for carbohydrate analysis. Wang discloses that the introduction of 9-aminopyrene-1,4,6,trisulonate for the CE-LIF of mono-and oligosaccharides observed a substantially higher molar absorptivity and quantum efficiency than most of the commonly used fluorphore carbohydrate derivatives and the presence of negatively charge functional groups appears to enhance the separation of mon0- and oligosaccharides. Pp. 431-432. Moreover, Wang discloses that the oligosaccharides were dissolved in water and then was mixed with ATPS and glacial acetic acid (solvent) and aqueous sodium cyanoborohydride, which was incubated and then diluted with a borate buffer and stored prior to CE separation, noting that the ionic strength of each buffer was adjusted so as to be approximately equal, in the acidic citric acid-phosphate buffer solutions, the electrophoretic mobility of th APTS-chitin oligosaccharides toward the outlet (anode is provide by negatively charged sulfonate groups under the negative applied voltage, i.e the migration sequence of analytes to the anode is based upon their apparent electrophoretic mobility. Registry information for DMSO disloses that its properties as a solvent are similar to

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

that of glacial acetic acid, the bcfs are very similar, they have high boiling points under the same conditions, they both accept at least one H, and their molar solubility is $>=1$ at the same pH ranges. Additionally, they are both utilized in the same reactions and thus their interchangeabilty would be obvious to one of ordinary skill in the art at the time of the invention. It would have been obvious to utilize 9-aminopyrene-1,4,6-trisulfonate (it would be in acid form in the reaction as it is in water) as the derivating agent capable of forming one or more fluorescing carbohydrate derivatives that are detectable by laser-induced fluorescence and the citric acid-phosphate buffer as taught by Wang in the composition of Charkoudian for the explicit reasons set forth in Wang.

Claims 1-8 meet the criteria set out in PCT Article 33(4), and thus there is industrial applicability because the subject matter claimed can be made or used in industry.